CHAUDHURI, H.

A DESCRIPTION OF COLLETOTRICHUM BIOLOGICUM, NOV. SP., AND OBSERVATIONS ON THE OCCURRENCE OF A SALTATION IN THE SPECIES.

IMPERIAL BUREAU OF MYCOLOGY

[Annals of Botany, Vol. XXXVIII. No. CLII. October, 1924.] Printed in England.

A Description of Colletotrichum biologicum, nov. sp., and Observations on the Occurrence of a Saltation in the Species.

BY

H. CHAUDHURI, PH.D. Lond., M.Sc. Cal., D.I.C.

(*Department of Botany, University of Panjab.*)

With Plate XVIII and seven Figures in the Text.

IN early October 1921 a fungus was collected from the dying stalks of potatoes in the garden of the Biologische Reichsanstalt für Forst- und Landwirtschaft at Dahlem-Berlin. The presence of the fungus was shown by a few black sclerotia on the surface of the stem, but just below the epidermis the sclerotia were very abundant. On careful examination under the microscope, the sclerotia were seen to bear black setae; from the sclerotia culture was made on potato extract in agar, and a scanty mycelium rapidly developed, producing innumerable black sclerotia and a few conidia borne irregularly. These conidia were unicellular, and germinated at once, producing similar mycelia and conidia. Single-spore dilutions were made on cover-slips, which were inverted on potato extract agar. As soon as the hyphae from the single-spore culture grew out beyond the cover-slip they produced black sclerotia. Hanging-drop cultures in distilled water were studied; here, too, the spores, after germination, produced sclerotia (as in Text-figs. 2 and 3), but prior to forming sclerotia the germ-tubes invariably produced first thick-walled dark chlamydospores (Text-fig. 1), and these on germinating produced later the sclerotia. The size of the conidia varies considerably; some are $10\mu \times 4\mu$, while others are $20\mu \times 6\mu$, the average being $14\mu \times 5\mu$. The sclerotia are roundish in form, but are somewhat conical at one end, like the fruit bodies of many of the Ascomycetes. A few stiff bristles are always borne by the sclerotia, as already stated. These characters naturally suggest that these structures are immature fruit bodies, but though the fungus has been growing for over seven months in different media, at various temperatures, no perithecia have yet been found; so that

[Annals of Botany, Vol. XXXVIII. No. CLII. October, 1924.]

they must be considered merely as sclerotia. The size of the mature sclerotium is 400–500μ × 300–360μ.

For five months this fungus produced only sclerotia and scanty conidia on a weak oatmeal agar, but when placed on strong oatmeal agar it suddenly started producing abundant mycelium and acervuli as well as sclerotia (Text-figs. 6 and 7). The conidia produced in the acervuli were narrower than the conidia borne free on the mycelium, measuring 22μ × 3·5μ on aver-

TEXT-FIGS. 1–7. 1. Germination of a conidium and development of a chlamydospore (*c*). 2. Young sclerotium developing. 3. Sclerotia more advanced. 4. *a*, sclerotia mature; *b*, seta from sclerotium. 5. Section of a sclerotium. 6. Acervulus and sclerotia. 7. Acervulus compressed beneath a cover-slip.

age. Sections were cut of the sclerotia in all stages of development, but no evidence of their ascocarp nature could be obtained.

None of the species of *Colletotrichum* hitherto described from Germany has the characters of this fungus. A *Colletotrichum* on potatoes has been reported by P. J. O'Gara from Salt Lake Valley, Utah, which he calls a new species, *Colletotrichum solanicolum*. The characteristics of this fungus given by him (7) much resemble those of the present form, from which, however, it differs in having two distinct types of conidia and much bigger sclerotia. From the similar sclerotia-producing species, viz. *C. tabificum*,

Pethybridge, and *C. atramentarium*, Taubenhaus, it is differentiated for the same reasons. There is very little difference between the three species of *Colletotrichum* described by O'Gara, Pethybridge, and Taubenhaus. Taubenhaus (11) says: 'It seems evident that *Phellomyces sclerotiophorus*, Frank, is the same as *Vermicularia atramentaria*, Berk. and Br., and also the same as *Colletotrichum solanicolum*, O'Gara. However, following the rule of priority, the fungus becomes *Colletotrichum atramentaria*, Tauben. Pethybridge (8) expresses doubt as to the reference of *Phellomyces* to *Colletotrichum* rather than to *Spondylocladium*. Pethybridge has considered in this paper *Rhizoctinia tabificum* of Hallier, which formed sclerotia, and which he found consisted of *Verticillium albo-atrum* (which certainly did not form sclerotia) and another fungus which really formed the sclerotia; this last fungus he described as *Colletotrichum tabificum* (Hallier *pro parte*). The descriptions given by Taubenhaus and O'Gara are similar, but comparing these with the description given by Pethybridge we find some difference. In the measurement of the conidiophore O'Gara gives 2–8 μ, whereas Pethybridge gives 20–30 μ. In cultures of the fungus here described the conidiophores are larger in most cases than those given by Pethybridge. It seems probable O'Gara's measurements apply to the segments of the conidiophores, hence the difference between him and Pethybridge. A strong reason against classing the present fungus as *C. tabificum*, Pethybridge, is the complete absence of any amethystine fluorescence, which Pethybridge describes as characteristic of his species.

Accordingly it appears most satisfactory to consider it as a new species, and the name *Colletotrichum biologicum* is suggested.

Colletotrichum biologicum, nov. sp., as grown in culture medium:

Acervulis in gelatina v. agaro *Solani tuberosi* gregariis atris 200–300 μ × 150 μ, interdum coalitis et ampliatis; basidiis cylindricis septatis 30–60 μ × 4–6 μ; conidiis oblongis rectis plerumque guttulatis 22 μ × 5 μ; setulis atris simplicibus rectis acuminatis usque ad 130 μ longis, ad basim 5 μ diam., ad apicem 2 μ diam. Mycelio hyalino septato, hyphis 3–4 μ diam.; conidiis oblongis, numerosis 14 μ × 5 μ; sclerotiis atris 400–500 μ × 300 to 360 μ; chlamydosporis atrofuligineis irregularibus; appressoriis atrofuligineis.

Behaviour of the Fungus on Different Media.

This fungus was grown in Coon's synthetic medium (4) in agar, on which, from inoculations from conidia, it produced a whitish mycelium bearing a few free conidia but at first no sclerotia (Pl. XVIII, Fig. 2 *a*). When inoculated, however, from the sclerotia it produced sclerotia only, and very few conidia (Pl. XVIII, Fig. 2 *b*). Later, in both cases, very minute black bodies somewhat bigger than the chlamydospores were formed.

Further experiments were carried out to determine the behaviour of the fungus towards maltose and asparagin. M/50 maltose agar and M/50 asparagin agar were inoculated with conidia and also with sclerotia. On asparagin agar the conidia produced a pure white mycelium but no sclerotia for a long time, whereas on maltose agar, under the same conditions, very little mycelium but numerous sclerotia were formed (Pl. XVIII, Fig. 3 *a*, &c.). When, however, these media were inoculated from sclerotia instead of conidia, the asparagin agar medium produced a few sclerotia with at first a dark and later a white mycelium instead of the wholly white mycelium produced on asparagin from conidia (Pl. XVIII, Fig. 4 *b*). Inoculation from sclerotia on the maltose agar medium produced a meagre mycelium with innumerable black sclerotia as before. The meagre production of conidia with glucose differs from Stevens's (10) result in his experiments with *Helminthosporium*, and the production of dark-coloured mycelium by asparagin and not by maltose is in opposition to his results ; and from the experiments carried on, which will be described under the section of colour production, it will be seen that it is more of a question of temperature than of medium.

Saltation.

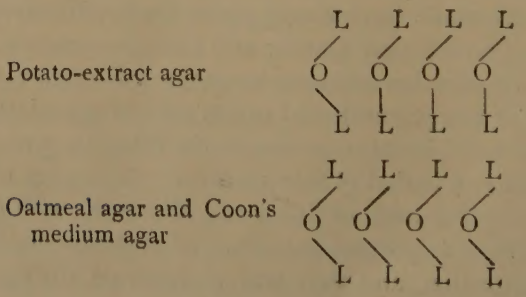
It grew well on potato-mush agar, oatmeal agar, Coon's medium, and various other food materials. This fungus was regularly grown on agar slopes of potato mush and of oatmeal, and transferred to fresh media every fortnight, the old cultures being kept. After four months' culture on oatmeal agar one tube developed a mycelium with pinkish colour, and from that a number of plates on Coon's medium in agar and on oatmeal agar were inoculated. Cultures were also made from the parent tube of the saltant, but no second saltation was found. Inoculations from the saltant were mostly started from single fragments of mycelium. A small portion of the agar medium with the growing zone of the mycelium was taken out with a scalpel, placed and pressed on a glass slide, examined under the microscope, and a single fragment removed for inoculation by means of a platinum needle, all being done under aseptic conditions. From these inoculations there developed parti-coloured cultures—portions of the mycelium being white and bearing normal large sclerotia in concentric rings, while other portions of the cultures in wedgelike form were pinkish and bore small black sclerotia arranged in radiating lines (Pl. XVIII, Fig. 1). From these cultures fresh inoculations were made on Coon's medium in agar, both from the ordinary normal large sclerotia and the smaller ones from the pinkish mycelium. The ordinary large sclerotia when transferred to fresh media produced only the larger ones (Pl. XVIII, Fig. 2). The smaller ones, however, developed a pink mycelium bearing at first only small sclerotia, but later producing whitish mycelium on which large sclerotia developed.

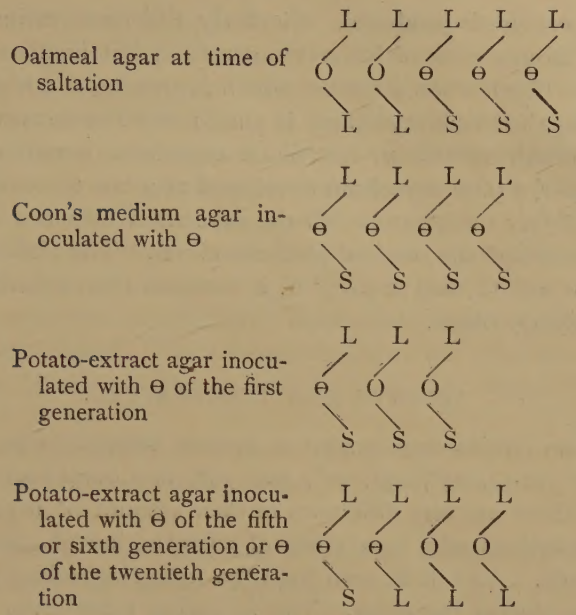
On oatmeal-agar medium the two strains behave as on Coon's medium, but more mycelial growth with free conidia is produced and many acervuli are developed. On potato extract, however, the two strains cannot be differentiated; when the smaller ones are inoculated into potato-extract agar or potato-mush agar, neither the coloured hyphae nor smaller sclerotia in radial rows are produced, but only large sclerotia in concentric rings. When, however, several generations of the new strain have been grown in Coon's medium in agar or on oatmeal agar—in which coloured mycelium and radiating sclerotia are always produced—and it is then transferred to potato extract agar, only a few sclerotia in radiating lines are produced on a very faintly coloured mycelium. Continued growth, however, on potato extract in agar for a couple of new generations leads to the complete loss of colour and the production of smaller radiating sclerotia. The behaviour of the saltant is never perpetuated in potato-extract agar; after growing the saltant for over twenty generations in Coon's medium, when it was put back on potato-mush agar and carried through a couple of generations all traces of the saltant were lost for good. Mutation in *Colletotrichum gleosporioides* has been mentioned by Burger (3). In his plate cultures of certain isolations of *C. gleosporioides* he found parts of the mycelium usually in wedge-shaped or fan-like areas showing a different colour from that of the rest of the growth. These variations were continued when transfers were made from the modified areas. Burger's *Colletotrichum* constantly gave off new types under natural as well as artificial conditions, and as these variations were more or less permanent they have been regarded as mutations. Mention of constantly mutating species, like Burger's, has been made by various workers, viz. Egerton, Dastur, Stevens, and others. Egerton (6), working with *Glomerella rufomaculans*, found permanent variations in a number of cases which may be looked upon as saltation. Dastur (5) found his original cultures of *Gleosporium piperatum* breaking suddenly into newer strains, and also that the mutation was a common phenomenon only in strains that were recently derived from the perithecia, for he observed no sudden variations from conidial strain. Stevens (10), working with various species of *Helminthosporium*, found that certain strains of a colony growing on an agar plate differed more or less from the rest of the colony, and when fresh transfers were made to new agar plates these differences were usually maintained on succeeding agar plates; and this phenomenon, which Stevens called saltation, was of rather common occurrence. Stevens also lends support to Dastur's view of recent perithecial origin favouring variations. The only mutation that occurred in this case was in the fifteenth generation from the original culture after a period of four months. Reviewing mutations which occur less frequently, the work of Shear and Wood (9) with *Glomerella* is of interest. They found important variations or mutation suddenly occurring in the fourth generation, and thus was transmitted through a number of

generations, but other variations also occurred which have been less permanent and have been regarded as fluctuations. In a recent paper Blochwitz (1) reports an interesting case of mutation in *Aspergillus versicolor*, which has usually a green colour. On making gelatin cultures he found in one generation three plates which produced a perfectly blue coloration, and cultures from these plates gave the blue pure form through over forty generations. Though the old cultures had been kept and subcultures were always made, no other case of blue coloration had appeared. Discussing the colour production, he says it is due to two things: (1) colour in the membrane, and (2) colour in the cell contents. In his mutation there was no coloration in the membranes of the conidia or mycelium. The disappearance of colour he ascribes to old age or due to long asexual reproduction. But in both cases the changes are very gradual, whereas in his three plates the changes were sudden and have been regarded as mutations.

Dealing with cases in which saltation or mutation has occurred only once, as in the present fungus, Brierley (2), working with *Botrytis cinerea*, which normally produces only black sclerotia, found in one of his single-spore inoculations in a Petri dish a mycelium which formed only pale sclerotia, and this new form reproduced itself in culture. In the present case, though the fungus has been grown for nearly two years, no second change has occurred. The first change occurred in a race that had been under culture for more than four months. As the change is permanent and shows its distinctive characteristics even when grown with the original under the same conditions, it has been termed a saltant. *Colletotrichum biologicum* has thus given rise suddenly to a saltant or new strain, the original being constant in character and never producing small sclerotia, while the new strain can produce both large and small sclerotia according to external conditions, i.e. according to the nature of the medium on which it is grown.

The following table shows the result of putting the saltant on potato-mush agar from the first up to the twentieth generation. Here O stands for the old normal strain, and Θ stands for the newer strain that has arisen by mutation from the older one; L for large sclerotia and S for smaller sclerotia.




Oatmeal agar at time of saltation
L L L L L
O O ϴ ϴ ϴ
L L S S S
Coon's medium agar inoculated with ϴ
L L L L
ϴ ϴ ϴ ϴ
S S S S
Potato-extract agar inoculated with ϴ of the first generation
L L L
ϴ O O
S S S
Potato-extract agar inoculated with ϴ of the fifth or sixth generation or ϴ of the twentieth generation
L L L L
ϴ ϴ O O
S L L L

During the whole course of the experiment subcultures were made fortnightly and the older parent cultures were preserved. When the new strain had appeared a number of subcultures were made from the parent cultures, but in no case did the second newer strain develop. The newer strain had evidently arisen by a single mutation. It is absolutely necessary in connexion with the work on mutation to see that the cultures are started either from a single spore or conidium or a small portion of mycelium. In the present work cultures both from conidia and single fragments of mycelium were made. The new strain differs from the old one in producing coloured hyphae and also smaller sclerotia; these two strains were grown, like the original strain, on Coon's medium in agar, on M/50 maltose agar, and M/50 asparagin agar, to determine if they behaved differently. Inoculation from either into asparagin agar produces coloured hyphae and some sclerotia arranged in concentric rings (Pl. XVIII, Fig. 4 *a*), but from the smaller ones sclerotia arranged both in concentric and radiating lines are produced (Pl. XVIII, Fig. 4 *c*).

Production of Colour and its Relation to Temperature.

The new strain produces a very bright pinkish colour at low temperature, and a black colour at higher temperatures. When the black mycelial growth developed at a higher temperature is inoculated into a fresh medium which is incubated at a low temperature the pink colour characteristic of

the low temperature is produced. Similarly a black mycelium is produced if inoculated from a pink of lower temperature, but incubated at a higher temperature. Even when a culture which is growing at a high temperature, and producing the black mycelium, is placed at a low temperature, the new growth is pinkish in colour, the black mycelium remaining unchanged. When, however, a pink mycelium developed at a low temperature is transferred to a higher temperature, all the mycelium becomes black, i. e. the newly produced and the original pink mycelium. The pink colour becomes fainter above 20° C., and at 22·5° C. it becomes almost dark ; at 25° C. or over it is perfectly black.

Growth and Temperature.

These two strains were grown at various temperatures, viz. 12°, 14°, 16°, 18°, 20°, 22·5°, 25°, 27°, 30°, and 32°, in Coon's normal medium, to determine if there was any difference in their amount of mycelial 'spread'. Besides inoculations with two kinds of sclerotia, inoculations from conidia were also made. As will be seen from the following table, the amount of 'spread' was always the same. The following table gives the measurement of the diameter of colonies grown in Petri dishes for seven days at different temperatures :

Temp. C.	*Parent.*	*Saltant.*	*Large.*
12°	15 mm.	15 mm.	15 mm.
14°	20 ,,	20 ,,	20 ,,
16°	25 ,,	25 ,,	25 ,,
18°	28 ,,	28 ,,	28 ,,
20°	35 ,,	35 ,,	35 ,,
22·5°	40 ,,	40 ,,	40 ,,
25°	46 ,,	46 ,,	46 ,,
27°	52 ,,	52 ,,	52 ,,
30°	47 ,,	47 ,,	47 ,,
32°	nil.	nil.	nil.

Summary.

1. *Colletotrichum biologicum*, nov. sp., collected in Germany in early October 1921 from dying stalks of potatoes, is described.

2. Only innumerable sclerotia with black setae were found on it in nature.

3. The cultural characteristics of this fungus were studied on different media and under various environmental conditions.

4. On potato-mush agar, Coon's medium, and on weak oatmeal agar it produced large sclerotia in concentric rings, scanty mycelia and conidia, but on strong oatmeal agar it produced abundant mycelium with acervuli as well as sclerotia.

5. Asparagin increased mycelial growth, whereas maltose increased sclerotia production.

6. After four months' culture in the laboratory (16 generations) saltation occurred, for it suddenly produced a new strain with a coloured mycelium bearing not only the large sclerotia in concentric rings of the parent form, but also small sclerotia in radiating lines.

7. Though the original culture has been continued on oatmeal agar, the medium in which it mutated, and also on various other media, for nearly two years, no second change has appeared.

8. The saltation is permanent and keeps its characteristics on Coon's medium in agar, and on oatmeal agar. When grown, however, on potato-mush agar it soon loses completely its distinctive characteristics and becomes the same as the original. The loss is permanent, for when put back to oatmeal agar or Coon's medium it fails to show the characteristics of the saltant.

9. There is no difference between the two forms in relation between growth and the effect of temperature. The optimum for both strains was 27° C.

10. The production of different colours by the saltant has been found to be entirely dependent on temperature.

The greater part of the work was done in the laboratory of the Department of Plant Physiology and Plant Pathology of the Imperial College of Science and Technology, London, during 1921–2. The paper has been finally completed in the Botany Laboratory of the Panjab University. The writer is indebted to various persons for their kind help. His best thanks are due to Professor V. H. Blackman, F.R.S., for his constant interest and helpful suggestions. He is also indebted to Dr. E. J. Butler, of the Imperial Mycological Bureau, Kew, and to Mr. Ramsbottom, of the Natural History Museum, South Kensington, for their help in the determination of the fungus. He is further indebted to Mr. Ramsbottom for valuable assistance with the Latin description, and to Dr. Horne and Dr. Brown for their many suggestions and criticisms, and to Mr. Tooley for taking the photographs of the culture plates.

LAHORE.
October 1923.

Literature cited.

1. Blochwitz, A.: Eine allgemeine Versuche spontaner Verlustmutationen bei Schimmelpilzen. Ber. d. Deut. Bot. Gesell, xli, p. 205, 1923.
2. Brierley, W. B.: On a Form of *Botrytis cinerea* with Colourless Sclerotia. Phil. Trans. Roy. Soc., London, B., ccx, pp. 83–114, 1920.
3. Burger, O. F.: Variations in *Colletotrichum gleosporioides*. Journ. Agric. Res., xx, pp. 723–36, 1921.
4. Coon, G. H.: Factors involved in the Growth and Pycnidium Formation of *Plenodomus fuscomaculans*. Journ. Agric. Res., v, pp. 713–69, 1916.
5. Dastur, J. F: *Glomerella cingulata* and its Conidial Form *Gleosporium piperatum*. Ann. Appl. Biology, vi, pp. 245–68, 1920.
6. Egerton, C. W.: The Physiology and Development of some Anthracnoses. Bot. Gaz., xlv, pp. 367–408, 1908.
7. O'Gara, P. J.: On a New Species of *Colletotrichum* from Salt Lake, Utah. Mycologia, vii, pp. 38–41, 1915.
8. Pethybridge, G. H.: Some Fungi in Potatoes. Trans. British Mycol. Soc., 1918.
9. Shear and Wood: Studies of Fungus Parasites belonging to the Genus *Glomerella*. U.S. Bureau of Plant Industry, cclii, 1913.
10. Stevens, F. L.: *Helminthosporium* Foot Rot of Wheat. Bull. Nat. Hist. Survey, Illinois, xiv, 1922.
11. Taubenhaus, W.: Silver Scart. Mem. New York Bot. Gard., vi, p. 549.

Explanation of Plate XVIII.

Illustrating Dr. H. Chaudhuri's paper on *Colletotrichum biologicum*.

Fig. 1. Plate-culture showing 'sectorial' growth, the new strain appearing as fan-like dark masses.

Fig. 2 *a*. Conidial inoculation on Coon's normal agar; white mycelium only produced. Fig. 2 *b*. Sclerotial inoculation on Coon's normal agar; innumerable sclerotia and scanty mycelium produced.

Fig. 3 *a*. Conidial inoculation on asparagin agar. Fig. 3 *b*. The same on oatmeal agar; the white dots in the centre are acervuli. Fig. 3 *c*. Conidial inoculation on maltose agar.

Fig. 4 *a*. Culture on maltose agar with large sclerotia only. Fig. 4 *b*. Culture on asparagin agar producing both large and small sclerotia. Fig. 4 *c*. Culture on maltose agar producing both large and small sclerotia.

1.

2 a.

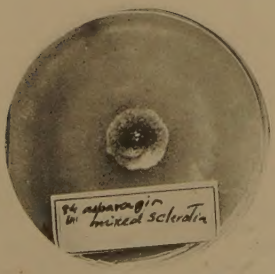
2 b.

a.

3. b.

c.

a.



b.

c.

4.

Huth coll.

CHAUDHURI—COLLETOTRICHUM.